Supplementary Figure Legends

Supplementary Figure 1. Recombinant clone analysis supporting NMD underlying removal of mRNA transcribed from transgene *hSOD1*^{E77X}, but not that from transgene *hSOD1*^{T116X}.

Both *hwtSOD1* and *hSOD1*^{E77X} or *hwtSOD1* and *hSOD1*^{T116X} transgenes were co-transfected at the same molar ratio into NIH/3T3 cells. Resulting mRNA was reverse-transcribed to cDNA and PCR-amplified using human-specific *SOD1* primers. The PCR product was cloned into plasmid pBluescript. Recombinant plasmid clones were randomly selected and individual identified by sequencing analysis. For the transgene *hSOD1*^{E77X}, we analyzed 38 clones from the group not treated with cycloheximide. We found that 32 clones represented hwtSOD1 and six clones represented hSOD1^{E77X}. The ratio of hSOD1^{E77X}/hwtSOD1 was 6/32 (18.8%). Among 57 clones from the cycloheximide-treated group, we identified 34 clones representing hwtSOD1 and 23 clones representing hSOD1^{E77X}. The ratio of hSOD1^{E77X}/hwtSOD1 was 45.5%.

For the transgene *hSOD1*^{T116X}, we analyzed 32 clones from the group not treated with cycloheximide. We found 22 clones representing hwtSOD1 and 10 clones representing hSOD1^{T116X}. The mRNA ratio of *hSOD1*^{T116X} to *hwtSOD1* was 9/20 (45.5%). Among 33 clones from the cycloheximide-treated group, we identified 23 clones representing hwtSOD1 and 10 clones representing hSOD1^{T116X}. The mRNA ratio of *hSOD1*^{T116X} to *hwtSOD1* was 10/23 (43.5%).

Supplementary Figure 2. NMD underlying removal of mutant *SOD1*^{K91X} mRNA. (a) Representative sequence electropherograms showing NMD of SOD1^{K91X}. Plasmid DNA containing cDNA of SOD1 with SOD1^{K91X} mutation was mixed with plasmid containing wild-type cDNA of SOD1 at 10 different ratios by amount, i.e. from 0.1 to 1.0 (mutant/wild-type). Mixed

DNA samples were used as templates for PCR-sequencing. The relative ratio of the peak height (T/A) was established by measuring the individual peak heights representing $SOD1^{K91X}$ (T) and wtSOD1 (A). The known molecular ratios (0.1 to 1.0) of the mutant/wild-type are labeled on the top of the sequence electropherograms of the sub-panels (a) to (j). Sub-panel (k) shows a sequence electropherogram of a stop codon (TAA) at codon 91, and (l) shows wtSOD1 at codon 91 when a single type of DNA was used. Sub-panels (m and n) show the sequence electropherograms of RT-PCR product of human SOD1 from NIH/3T3 cells transfected with equal molar ratio of $SOD1^{K91X}$/wtSOD1 plasmids; CT, cycloheximide-treated; NT, not treated with cycloheximide. Sub-panels (o) and (p) show the sequence electropherograms of RT-PCR product of human SOD1 from another cell line (NSC34) transfected with equal molar ratio of $SOD1^{K91X}$/wtSOD1 plasmids.

(b) A standard curve showing ratios of peak height (T/A) against known molecular ratios ($SOD1^{K91X}$/wtSOD1). Cyc- (+), cycloheximide-treated; Cyc- (-), not treated with cycloheximide.

(e) Relative steady-state mRNA levels of $SOD1^{K91X}$ in cell lines of NIH/3T3 and NSC34 compared with wtSOD1. Over 80% of mRNA transcribed from human $SOD1^{K91X}$ was eliminated, and inhibition of translation by cycloheximide significantly suppressed such elimination in both NIH/3T3 and NSC34 cell lines (Student's t-test, $P<0.001$).

Supplementary Figure 3. Sothern blot analysis of the $hSOD1^{T116X}$ mice.

Six $hSOD1^{T116X}$ transgene-positive mice identified by PCR were analyzed by Southern blot. Mouse tail DNA was digested with *Pst*I to completion, separated on a 0.8% agarose gel, transferred to a nylon membrane and hybridized with a human SOD1-specific probe isolated from intron 1 of human SOD1 gene. A 2.4kb human SOD1-specific band was detected, validating that

these mice are transgenic. A significantly increased dose in lane 4 (arrow) suggests that this mouse is possibly a homozygote, while the others are heterozygotes.

Supplementary Figure 4. Kaplan- Meier showing the cumulative survival of the hSOD1^{T116X}/hwtSOD1 mice.

The hSOD1^{T116X}-alone heterozygous mice (n>20) do not develop an ALS-like phenotype in their life time (>18 months) (blue). The hSOD1^{T116X}/hwtSOD1 mice (n=5) develop an ALS-like phenotype at the ages of 13-18 months [15.4 ± 0.90 (M ± SE), Log Rank test χ^2 =35.3, P<0.0001].

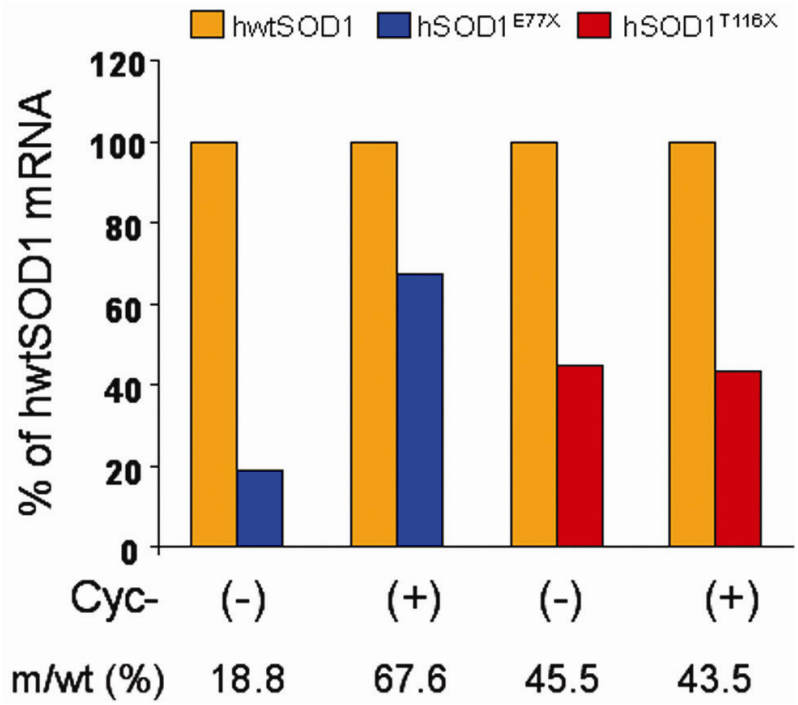
hwtSOD1
hSOD1E77X
hSOD1T116X
% of hwtSOD1 mRNA
120
100
80
60
40
20
0
Cyc-
(-)
(+)
(-)
(+)
m/wt (%)
18.8
67.6
45.5
43.5

Fig. S1

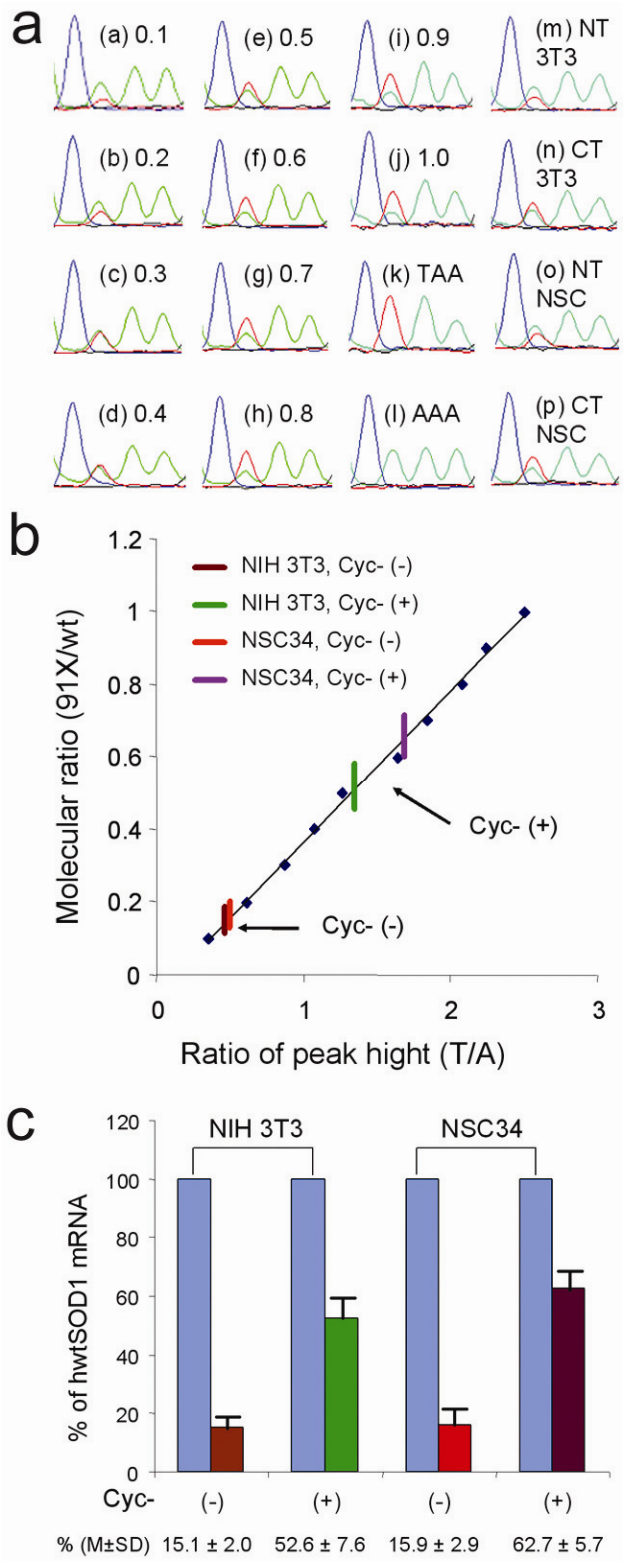
a
(a) 0.1
(e) 0.5
(i) 0.9
(m) NT 3T3
(b) 0.2
(f) 0.6
(j) 1.0
(n) CT 3T3
(c) 0.3
(g) 0.7
(k) TAA
(o) NT NSC
(d) 0.4
(h) 0.8
(l) AAA
(p) CT NSC
b
NIH 3T3, Cyc- (-)
NIH 3T3, Cyc- (+)
NSC34, Cyc- (-)
NSC34, Cyc- (+)
Molecular ratio (91X/wt)
Ratio of peak hight (T/A)
Cyc- (+)
Cyc- (-)
c
NIH 3T3
NSC34
% of hwtSOD1 mRNA
Cyc-
(-)
(+)
(-)
(+)
% (M±SD)
15.1 ± 2.0
52.6 ± 7.6
15.9 ± 2.9
62.7 ± 5.7

Fig. S2

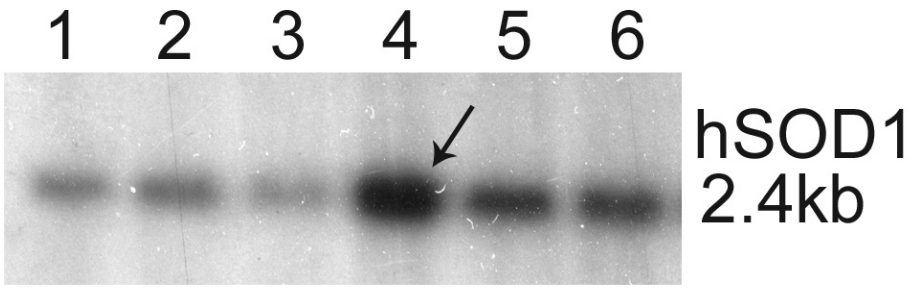

Fig. S3.

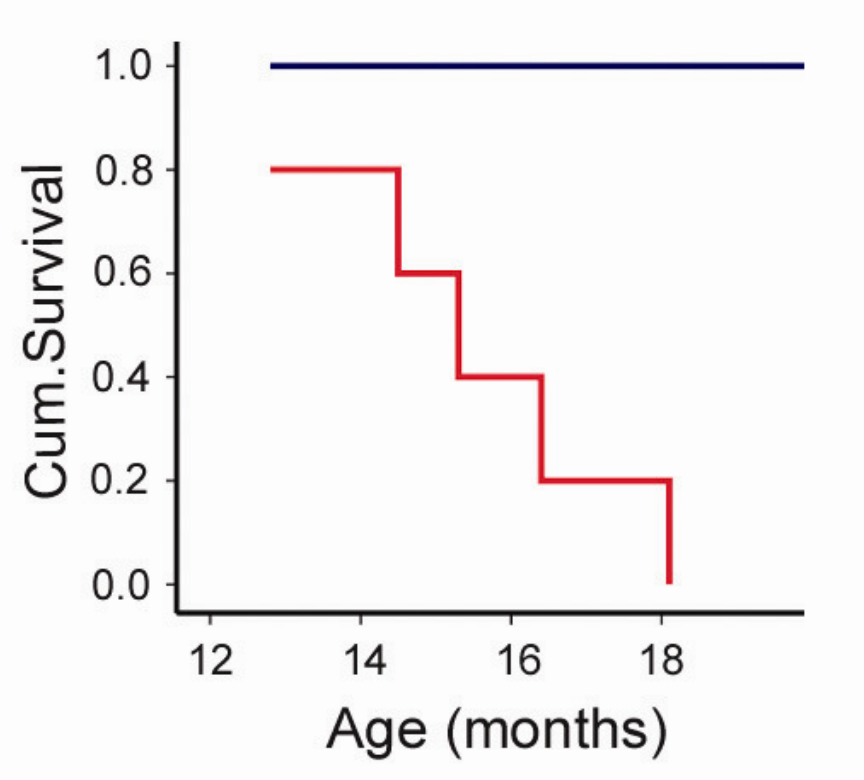

Fig. S4.